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# Blockade of Cardiac Sodium Channels by Lidocaine

## Single-Channel Analysis

Augustus O. Grant, Margaret A. Dietz, F. Roosevelt Gilliam III, and C. Frank Starmer

The mechanism of interaction of lidocaine with cardiac sodium channels during use-dependent block is not well defined. We examined the blockade of single cardiac sodium channels by lidocaine and its hydrophobic derivative RAD-242 in rabbit ventricular myocytes. Experiments were performed in cell-attached and inside-out patches. Use-dependent block was assessed with trains of ten 200-msec pulses with interpulse intervals of 500 msec and test potentials of  $-60$  to  $-40$  mV. Single-channel kinetics sometimes showed time-dependent change in the absence of drug. During exposure to  $80 \mu\text{M}$  lidocaine, use-dependent block during the trains was associated with a decrease in the average number of openings per step. At  $-60$  mV, mean open time was not significantly changed (control,  $1.4 \pm 0.6$  msec; lidocaine,  $1.2 \pm 0.3$  msec,  $p > 0.05$ ). Greater block developed during trains of 200-msec pulses compared with trains of 20-msec pulses at the same interpulse interval at test potentials during which openings were uncommon later than 20 msec ( $-50$  and  $-40$  mV). Prolonged bursts of channels showing slow-gating kinetics were observed both in control and the presence of  $80 \mu\text{M}$  lidocaine. However, lidocaine may decrease the late sodium current by altering the kinetics of slow gating. The hydrophobic lidocaine derivative RAD-242, which has a 10-fold greater lipid solubility than lidocaine, decreased the peak averaged current during pulse train stimulation by 60% without a change in the mean open time. Our results suggest that the major effect of lidocaine during use-dependent block involves the interaction with a nonconducting state of the sodium channel followed by a failure to open during subsequent depolarization. (*Circulation Research* 1989;65:1247-1262)

Lidocaine is the antiarrhythmic drug most frequently used for prophylaxis and treatment of postinfarction ventricular arrhythmias. The antiarrhythmic effects are exerted at concentrations 1/100 or less of that required to block the nerve action potential.<sup>1,2</sup> In both tissues, the drug is believed to exert its effect by blockade of the inward sodium current ( $I_{Na}$ ).<sup>3,4</sup> Upstroke velocity and macroscopic current measurements have shown that block is strongly dependent on the rate and pattern of stimulation.<sup>4</sup> The patterns of blockade have been interpreted by models based on variations of affinity with voltage-dependent channel state or of state-dependent access of drug to its binding site.<sup>3,5</sup> Recent ligand-binding studies suggest the presence of a stereospecific binding site for

lidocaine and other antiarrhythmic drugs on the sodium channel protein.<sup>6,7</sup>

Whole-cell  $I_{Na}$  and binding studies do not provide direct information about the binding of the drug to defined states of the channel and the relative potencies of intracellular and extracellular drug application. Recent recording of single sodium channel current in cardiac myocytes offer a number of possibilities for analysis of the molecular basis of the blocking action of lidocaine.<sup>8-12</sup> The probability that a channel may open in response to depolarization, the amplitude of the current of a single channel at a given test voltage (conductance), and the distribution of channel open times can all be measured directly. Known concentrations of drug can be applied to either the intracellular or extracellular membrane surface. The whole-cell sodium current is related to the underlying single channel events according to the equation:  $I_{Na} = NP_o i$ , where  $N$  is the number of channels,  $P_o$  is the probability of a channel being open, and  $i$  is the unitary current of a sodium channel. Therefore, during use-dependent inhibition, lidocaine and other antiarrhythmic drugs may reduce  $I_{Na}$  by a reduction of  $N$ , of  $P_o$ , of  $i$ , or of

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combinations of these. The analysis of single-channel current can be used to distinguish these possibilities.

Few studies have exploited the opportunity afforded by single-channel recording to define the basic action of lidocaine. Nilius et al.<sup>13</sup> observed a 50% reduction in mean open time when 5  $\mu$ M lidocaine was applied to the internal surface of inside-out membrane patches. This result is at variance with whole-cell voltage-clamp experiments, which suggest primarily inactivated-state block by lidocaine and similar potency for intracellular and extracellular application of lidocaine.<sup>4,14</sup> Their report also suggested a  $K_d$  for the reduction of  $P_o$  of only 2  $\mu$ M. A preliminary report by Kohlhardt et al.<sup>15</sup> suggests that lidocaine was ineffective in reduction of the open time in inside-out patches of neonatal cardiac myocytes in which inactivation was removed by the diphenylpiperazinyldole derivative DPI.

We have examined the mechanism of block of single cardiac sodium channels by lidocaine and its hydrophobic derivative RAD-242. We performed a majority of our experiments in the cell-attached configuration because single-channel kinetics are more stable in this configuration and may parallel the normal application of the drug more closely.<sup>16</sup> We used trains of pulses that permitted analysis of the dependence of block on channel use, duration of the depolarizing pulse, and test voltage. Our initial results show that the major effect of lidocaine is a reduction of  $P_o$ . In contrast to the results of Nilius et al.,<sup>13</sup> we observed weak open channel-blocking properties.

### Materials and Methods

#### *Cell Preparation, Solutions, and Electrodes*

Single ventricular myocytes were prepared from rabbit ventricles with a previously described enzyme dissociation procedure.<sup>12</sup> All steps requiring the use of Kraft-Bruhe medium were omitted. The tissue and isolated cells were maintained at 37° C throughout the isolation procedure. After enzyme digestion of the heart, the atria, right ventricular free wall, and left ventricle including the septum were separated, and single cells were prepared from each region. The isolated myocytes were stored in a CO<sub>2</sub> incubator at 37° C. They were used within 12 hours of isolation.

For cell-attached recordings, the isolated myocytes were superfused with a high external potassium solution of the following composition (mM): KCl 140, NaCl 10, MgCl<sub>2</sub> 5, EGTA 0.05, HEPES 5, and glucose 10. The pH was adjusted to pH 7.4 with KOH. The high external potassium solution depolarized the cells to approximately 0 mV. Therefore, all membrane potentials were quoted as absolute voltage levels. Micropipettes were filled with an external solution of the following composition (mM): NaCl 152, KCl 5.4, MgCl<sub>2</sub> 2.4, CaCl<sub>2</sub> 0.02, HEPES 5, and glucose 11. When we initiated our single-

channel studies, we used an external calcium concentration of 2.7 mM and could detect no change of single-channel current amplitude with depolarization.<sup>8</sup> Our preliminary drug studies (of the antiarrhythmic drug quinidine) using 1 mM extracellular calcium concentration still showed marked rectification. Therefore, we performed these studies with low external calcium in which this effect was no longer evident. For excised inside-out patches, the high potassium external solution bathed the inner membrane surface. Lidocaine hydrochloride was obtained from Astra Pharmaceutical Products, Worcester, Massachusetts. The lidocaine derivative RAD-242<sup>17</sup> was provided by Dr. R. Sandberg of Astra Alab AB, Sodertalje, Sweden.

Micropipettes were fabricated from 1.6 mm o.d. capillary tubing of aluminosilicate (A-M Systems, Everett, Washington) or borosilicate glass (type 7052, Garner Glass, Clairmont, California, or type N51A, Drummund Scientific, Bromall, Pennsylvania). Micropipettes were coated with Sylgard 184 (Dow Corning, Midland, Michigan) and fire-polished immediately before use.

#### *Recording Techniques*

We measured single-channel currents with an EPC 7 patch-clamp amplifier (List Electronics, Darmstadt, FRG). Each microelectrode was coupled to the input of the amplifier by a silver/silver chloride wire coated up to its tip with Teflon. A bath reference electrode was fashioned from a silver/silver chloride pellet and a 3% agar gel of the pipette filling solution. Both electrodes were stored in the pipette solution when not in use. Offset voltages were stable and typically 1–3 mV. These were nulled before obtaining recordings. Currents were recorded on an analog tape recorder (model 4DS, Racal Recorders, Vienna, Virginia) at 3 3/4 in/sec ("wideband width 1"; DC, 2.5 kHz). The currents were filtered at a corner frequency of 1.5 kHz during off-line analysis with an 8-pole Bessel filter (model 902LPF-1, Frequency Devices, Haverhill, Massachusetts). The voltage command pulses were provided by an IBM XT personal computer equipped with an A/D-D/A interface (TL1-I interface with Labmaster boards, Axon Instruments, Burlingame, California). The pulses were filtered at a corner frequency of 4 kHz before application to the input of the patch-clamp amplifier.

#### *Experimental Protocol*

An aliquot of cells was allowed to settle on the laminin-coated base of the recording chamber for 15 minutes. Superfusion was then initiated with the high potassium external solution. Experiments were performed on the rod-like cells with clear striations that remained adherent to the base of the recording chamber. Gigaohm seals were obtained according to the method of Hamill et al.<sup>18</sup> For cell-attached recording, the membrane was hyperpolarized to a holding potential of –120 mV. The capacitive tran-

sient during step changes in potential was partially compensated with analog circuitry. For groups of voltage steps in which few nulls were evident, 10–20 voltage steps of polarity opposite to that used to elicit currents were also applied. During analysis, these steps were averaged and used for residual capacitive and leakage current subtraction.

We used two basic stimulation protocols in these studies. The first was designed to examine the use-dependent inhibition of the sodium channel current by lidocaine. Trains of ten 200-msec pulses with an interpulse interval of 500 msec were applied. Each pulse train was followed by a recovery interval of 3 seconds. We usually applied a total of 60–80 such trains, giving a total of 600–800 voltage steps. A majority of the voltage steps was applied to test potentials of  $-60$  or  $-40$  mV. Additional experiments were also done to a test potential of  $-50$  mV.

The second series of pulse protocols was designed to examine the effect of pulse duration and of membrane potential on the block produced by lidocaine. Eighty to 100 voltage steps of 200-msec duration were applied at an interpulse interval of 500 msec. These were then followed by eighty to one hundred 20-msec pulses with the same interpulse interval (500 msec). To check for time-dependent “rundown” (the decline in the apparent number of functioning channels in the patch), the sequence of 200-msec pulses was then repeated. A total of 240–300 steps were, therefore, performed at each potential. We used test potentials of  $-70$ ,  $-60$ ,  $-50$ , and  $-40$  mV for the pulses in the train. We report the data if we completed three or more of these potentials.

The usual approach was to obtain control data in one patch. A second membrane patch was then obtained on the same cell or a cell isolated from the same ventricle. Eighty or 160  $\mu$ M lidocaine was now included in the micropipette solution. The cells were superfused for 15 minutes with the high potassium solution containing the same concentration of lidocaine present in the micropipette before obtaining a second seal. In a few experiments the cells were superfused with lidocaine-containing solution for 10 minutes after control data had been obtained. Solenoid valves (The Lee Company, Westbrook, Connecticut) located close to the perfusion chamber permitted rapid changes of solution. The protocols were then repeated on the same patch during the continued exposure to lidocaine. In these experiments, no lidocaine would be present in the micropipette. All experiments were performed at  $18^\circ$  C.

#### Data Analysis

Currents were digitized at 10 kHz. The analysis was done off line on a MicroVax II/GPX workstation (Digital Equipment, Merrimack, New Hampshire) with software developed in our laboratory. The records were first scanned, and all steps without events were collected. These were averaged and used to remove the residual capacitive and

leakage currents. At potentials where very few (less than five) nulls were evident, current responses to pulses of the same magnitude, but opposite polarity, were used for leakage and capacitive transient subtraction. In the next step of analysis, all data points at a given potential were used to form a histogram (Figure 1, panels A and B). Care was taken to exclude any points that clearly represented residual capacitive transient. The histogram was multimodal with the largest peak representing the baseline. The next peak represented the opening of single channels. We used the difference between these two peaks to define the open level of a single channel. At some potentials (e.g.,  $-70$  mV), very few events were observed, and the histogram approach was not suitable to define the single-channel current level. Under these circumstances, the records were scanned for clearly resolved events. These were averaged and used to define the single-channel current level.

We used an automatic threshold detection scheme to identify events.<sup>19</sup> The threshold was usually set at 0.5 times the amplitude of a single event. Despite a good signal-to-noise ratio, we found it important to review every event detected in each step. On occasions, a second diminished-conductance level was present. The second and third tracings in panel D of Figure 1 and second tracing in panel E are examples. The low amplitude events in panel D were of long duration with peaks near the threshold. This led to multiple crossings of the threshold and the detection of a large number of brief “false events.” Two events are evident at times after the arrow in the second tracing of panel B, yet the threshold detection scheme identified 41 events. The low-level events that posed the most problems appear to be subconductances of the normal state of the sodium channel that occurred at late times. This is suggested by the events in the third tracing of panel D in which a brief opening to the full conductance level interrupts the subconductance opening. Support of this idea is illustrated in panel E in which a run of three consecutive steps with prolonged bursting was observed. The events appear to arise from a single channel. Yet during the second step, the conductance level was reduced to about one third of normal. Steps showing such late low-level conductances were eliminated from analysis. The recently described low conductance cardiac sodium channels could pose a similar problem for the automatic detection scheme.<sup>20</sup> For overlapping events, we used a random scheme to assign openings and closings. The computer randomly assigns a given closure with one of the preceding openings. We have performed an extensive series of simulation that shows that this method provides nonbiased estimates of open times (J. Hurwitz et al, submitted manuscript).

We used the maximum likelihood estimate to characterize the channel-closing kinetics.<sup>19</sup> The

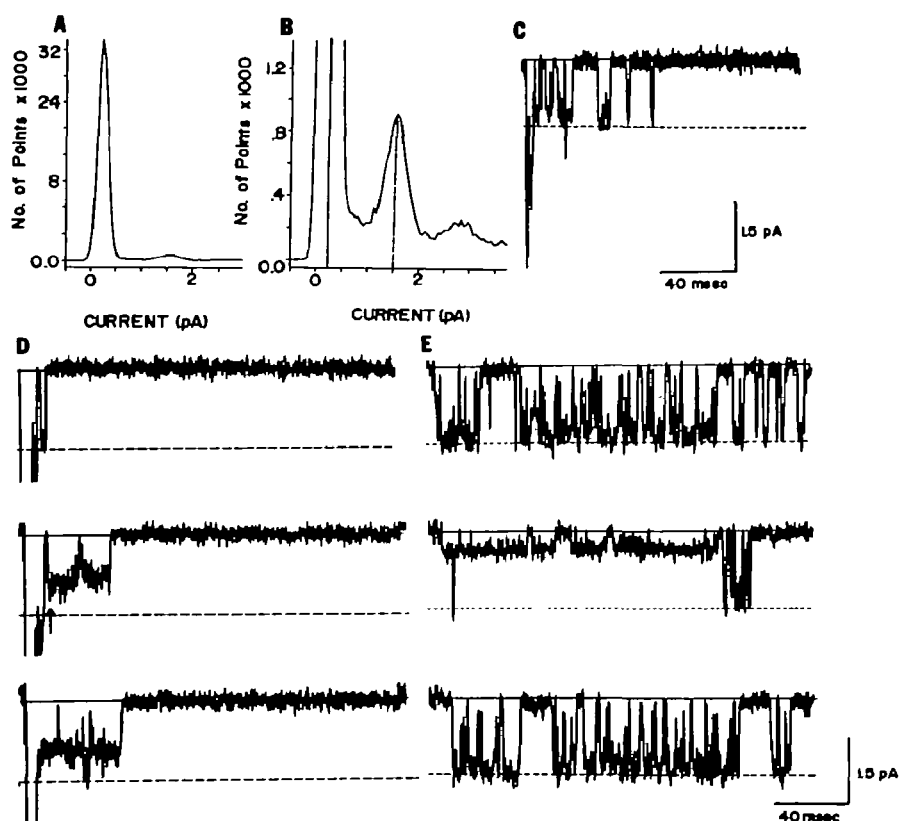


FIGURE 1. Graphs and tracings showing scheme for detection of single-channel events. Panel A: Histogram formed from all the data points from 100 capacitive-transient and leakage-subtracted sweeps. At least two peaks are evident; one peak represents the baseline, and the second peak represents the single channel open level. Panel B: Expansion of the lower half of panel A. The second peak is now clearly visible. The distance between the solid vertical line (baseline) and the vertical broken line (unit open level) gives the amplitude of the single channel event (1.45 pA in this experiment). The units on the ordinate are in thousands of events. Panel C: Initial segment of one of the sweeps used to form the histogram. The single-channel current amplitude is shown as the broken line. Events are detected by setting the threshold one half of the distance between the baseline determined for each sweep and by the single-channel current level. Panel D: One of the confounding problems with the automatic detection scheme. The three sweeps in this panel were obtained from a cell-attached patch during voltage steps from  $-120$  to  $-60$  mV. The first sweep shows an initial surge of sodium current followed by a single brief opening of normal amplitude. The second sweep shows two late long-duration events of reduced amplitude (to the right of the arrow). These two events crossed the threshold repeatedly such that a total of 41 "false events" were detected. The subsequent sweep (third sweep) shows similar late low-amplitude events, but there is a brief opening to the full current level. This suggests that the openings of reduced amplitude are subconductance states of the sodium channel. Panel E: Three sweeps from another patch. The three sweeps are consecutive and show one channel bursting for most of the 200-msec duration of each sweep. However, the burst during the second sweep is at a reduced level for most of the duration of the sweep.

method assumes a single exponential distribution of open times, and the closing rate was then the reciprocal of the arithmetic mean of the open time. We also measured the closing rate from the weighted least-squares fit to histograms of the open times (bin width, four sampling intervals).<sup>21</sup> The fit of the distribution by a single exponent was assessed by a minimum  $\chi^2$  procedure. A single exponent usually provided a good fit.<sup>22</sup> We have performed simulations that show that the time constant from the histogram is less reliable than the arithmetic mean when the number of events is small (J. Hurwitz et al, submitted manuscript). The data from the pre-

sent studies confirmed the simulation. The results of the two methods converged when the number of events was large. Since lidocaine decreased the number of openings, we used the means of the open times for comparisons with control.

Further analysis of the data obtained with specific protocols is deferred to the results. Data are quoted as the mean  $\pm$  sample SD. For channel open times that are exponentially distributed, the mean and sample standard deviation should be the same.<sup>19</sup> In some experiments the standard deviation was slightly greater than the mean. Therefore, the range includes negative values of open time that are not physically realizable. As pointed out by Colquhoun and

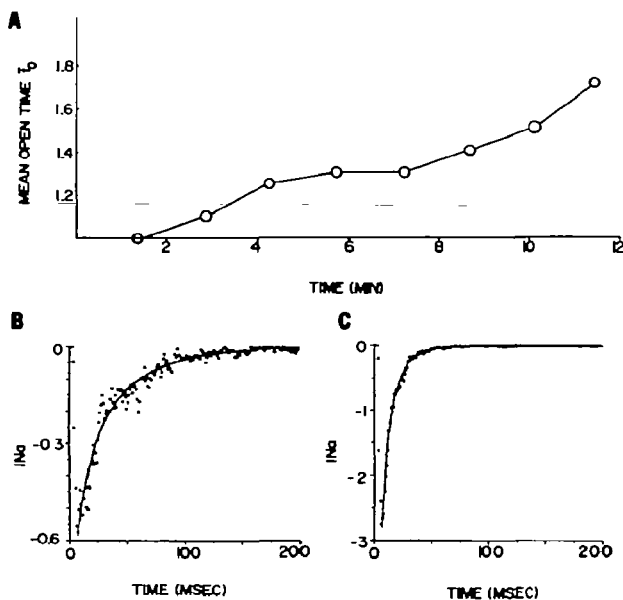


FIGURE 2. Graphs showing time-dependent changes in sodium-channel kinetics. The data in panels A, B, and C were obtained in a cell-attached membrane patch. The mean open time (msec) was determined from a group of 100 voltage steps at the time points indicated. Panel A: Progressive increases of mean open time. Panel B: Averaged current during the first series of pulses. Panel C: Averaged current during eighth series of pulses. The ordinate in panels B and C are in units of single-channel events (unitary amplitude, 1.9 pA). The continuous lines are least-squares fit with double exponents to the relaxation phase of the average current. The relaxation time constants were 14.9 and 50 in panel B and 6.9 and 0.15 msec in panel C.

Sigworth,<sup>19</sup> likelihood intervals are more appropriate. However, their estimation is computationally formidable, so we elected to use the sample standard deviation.

## Results

### Stability of Channel Kinetics

In the evaluation of the kinetic parameters of single sodium channels, it was important to establish that they were stable over the observation period. In physiological experiments, it is possible to repeat the control observations after a variable such as test voltage is varied. Evaluation of drug effects does not lend itself readily to such controls; hence, the issue of stability assumes greater importance. We observed time-dependent changes in single-channel opening probability and open time in a number of experiments.

Channel mean open time showed time-dependent change. In panel A of Figure 2, the mean open time in a cell-attached patch increased progressively from 1 to 1.8 msec during steps from the holding potential of  $-120$  mV to  $-60$  mV over a 12-minute observation period. Each time point represents the

mean open time of events in 100 steps. The patch had at least five channels, and each group of 100 steps had 1,925–2,438 events. Therefore, the error in the estimate of the mean open times was quite small. We are not certain of the reason for the change in mean open times. A decrease in the cell membrane potential could make the effective test voltage more positive. As channel mean open time increases with depolarization positive to  $-60$  mV, the decline in effective voltage could account for the increase in mean open time.<sup>12</sup> However, we could not detect a corresponding change in single-channel conductance. A spontaneous separation of the microelectrode from the cell followed by the formation of an inside-out patch in  $K_2$  EGTA containing bathing solution could account for the change in closing kinetics. Single-channel mean open times increase after patch excision and are less stable than in the cell-attached configuration.<sup>12,16,23,24</sup> The averaged current for the first and eighth time points in panel A are illustrated in panels B and C. In both cases, the current relaxed as double exponents. However, the fast time constants were 14.9 and 6.9 msec, and the slow time constants were 50 and 15 msec for the early and late data set. This suggests that the parameters that characterize channel gating shifted along the voltage axis with time. In three of 17 experiments using a similar protocol, we observed progressive changes in mean open times of 30% or greater. Changes in open times such as this could vitiate genuine drug effects.

The extent of the time-dependent decrease in the number of openings during repetitive pulses to the same potential varied between patches. When the decrease was clearly evident to the experimenter viewing the oscilloscope tracings, the experiment was terminated. Such experiments were not analyzed. In other experiments, the change was only evident after detailed analysis. It was not possible to distinguish unequivocally between a time-dependent decrease in the number of functioning channels or a time-dependent decrease in the probability that a channel will open. Because experiments in which the change in the number of openings was most marked were excluded from analysis, it would not be meaningful to give summary values of the frequency with which the decline in the number of openings occurred.

We do not think the types of time-dependent changes that we observed are unique to our recording conditions. Their occurrence is implicit in the description of the methods in other studies of the sodium channel.<sup>10,11,23</sup> In fact, it is a surprise when they are not discussed in some studies of drug effects (e.g., Reference 13). As far as possible, we used protocols that could take such time-dependent changes into account.

### Use-Dependent Block by Lidocaine

We examined the use-dependent block of single cardiac sodium channels by  $80 \mu\text{M}$  lidocaine in five

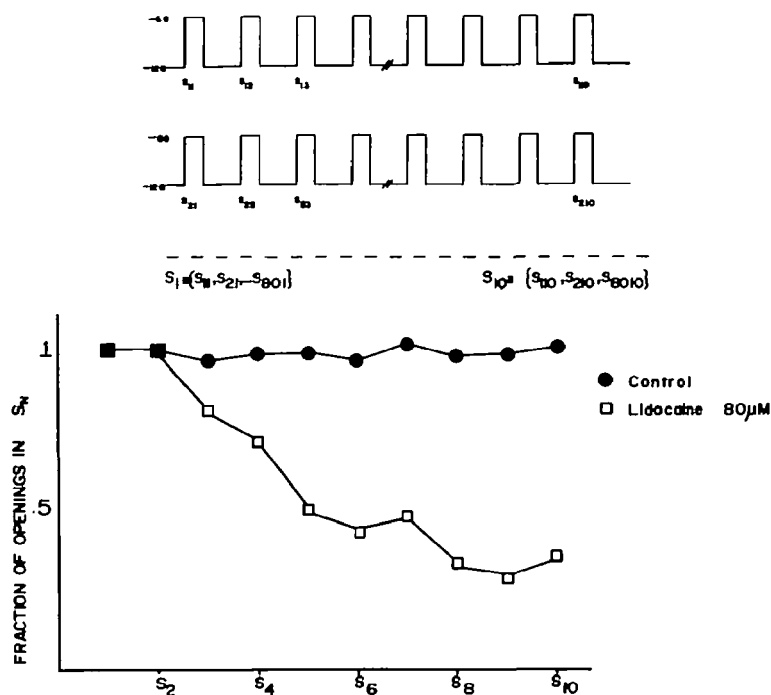


FIGURE 3. Diagrams and graph showing use-dependent inhibition of sodium channel by  $80 \mu\text{M}$  lidocaine. The upper part of the figure shows the pulse paradigm. Currents were elicited by trains of ten 200-msec pulses from a holding potential of  $-120$  to  $-60$  mV. A total of 80 trains was performed. The pulses were segregated into groups according to their sequence in their respective trains ( $S_1$ , the set of first pulses of all trains). The number of openings in each group has been normalized by that in  $S_1$  and plotted on the ordinate; the group set ( $S_1, S_2, \dots$ ) is plotted on the abscissa. The data during control and lidocaine exposure were obtained in separate patches. The control patch contained at least seven channels; the patch during lidocaine exposure contained five channels.

pairs of patches. One of each pair served as the control; the second was exposed to drug. Sixty to 80 trains of 200-msec pulses were applied with 500 msec between the pulses. A rest interval of 3 seconds separated the trains. The  $n$ th pulse of each pulse train is grouped across 60–80 trains. In this manner, any time-dependent changes should affect the groups in a similar manner. During control, the average number of open events per step was stable over 10 pulses during the train. There was no difference in open time between the events in the first and tenth pulses of the trains. This indicated that the 500-msec interval between pulses was enough time for normal recovery from inactivation. Open times appear to be independent of channel history. The data from the control patch illustrated in Figure 3 contained at least seven channels, and no nulls were observed in a total of 800 steps to  $-60$  mV. During exposure to lidocaine, the averaged number of openings declined progressively during the train in a patch containing five channels. This was associated with an increase in the number of nulls between the groups of first pulses and of the tenth pulses. The increase in the number of nulls should reflect blocked channels because the interval between pulses was enough to permit recovery of inactivated unblocked channels. The null sweeps tended to occur in runs. However, we elected not to do any quantitative analysis on the length of the runs because, during pulse-train stimulation, the pulses within a train are not equivalent.

The mean open times did not change significantly between the first and the tenth pulse. The means for the tenth pulse represent a smaller number of events because of the progressive blockade during the trains. We have summarized the results of all five pairs of

experiments in Table 1. Use-dependent inhibition reflects a decrease in the number of open events rather than a change in the duration of openings (the question of whether open time is generally changed by drug is discussed in a later section).

#### Relation Between Pulse Duration and Cumulative Block

Upstroke velocity and voltage-clamp experiments show that lidocaine block develops during depolarizing pulses over a time frame of hundreds of milliseconds.<sup>25,26</sup> It is not clear if the block occurring at late times during a voltage step, after the initial sodium current surge, is dependent on abbreviating or abolishing late events or is independent of open events. In general, single-channel recordings provide better definition than whole-cell recordings of the few events that occur at late times. However, rundown over time and the need for a stable seal of long duration limit the usefulness of the technique in providing detailed kinetic data.

As a compromise, we compared the extent of block produced by 20- versus 200-msec pulses applied to the same potential. We anticipated greater block with the 200-msec pulse; therefore, the power of the experiment was increased by applying the 200-msec pulse first. Rundown would decrease the number of events in the subsequent 20-msec pulse and negate the expected results. As a further control, the 200-msec sequence was repeated after a sequence of 20-msec steps. We analyzed the experiment if data were obtained at three or more potentials. This meant the acquisition of data from a minimum of 900 steps during drug exposure and the same number from at least one drug-free patch.

TABLE 1. Effect of Lidocaine on Single Sodium Channels During Pulse-Train Stimulation

Pairs of patches (X, Y)	Pulse order	V <sub>T</sub> (mV)	Control			Lidocaine		
			Av	Fn	t <sub>o</sub> (msec)	Av	Fn	t <sub>o</sub> (msec)
b,a	S <sub>1</sub>	-60	1	...	2.2±2.1	1	0.4	1.5±1.4
	S <sub>10</sub>	...	1.01	...	2.2±1.9	0.35	0.7	1.2±1.1
e,f	S <sub>1</sub>	-60	1	...	1.6±1.5	1	0.43	1.3±1.2
	S <sub>10</sub>	...	0.99	...	1.7±1.4	0.85	0.49	1.3±1.2
g,h	S <sub>1</sub>	-60	1	...	1.3±1.3	1	0.05	1.2±1.2
	S <sub>10</sub>	...	0.94	...	1.4±1.5	0.62	0.2	0.94±1
j,k	S <sub>1</sub>	-50	1	...	1±1	1	0.18	1±0.9
	S <sub>10</sub>	...	0.99	...	1.1±1	0.52	0.44	0.9±0.7
l,m	S <sub>1</sub>	-40	1	0.5	0.89±0.9	1	0.18	0.9±0.8
	S <sub>10</sub>	...	1.3	0.45	0.9±1	0.29	0.6	0.9±1.2

V<sub>T</sub>, test potential; Av, average number of openings in each set, S<sub>n</sub>, divided by the number in the set S<sub>1</sub>; Fn, fraction of null sweeps observed; t<sub>o</sub>, mean open time (mean±SD); S<sub>1</sub> and S<sub>10</sub>, pulse orders segregated into groups according to their sequence in their respective trains (e.g., S<sub>1</sub> is the set of first pulses of all trains). The number of channels in different patches affects Fn. However, the trend for Fn to increase between S<sub>1</sub> and S<sub>10</sub> was observed in all preparations.

Because of the difficulty in maintaining stable recordings during such prolonged periods, the number of successful experiments was small. The data obtained in five sets of experiments from eight patches (three controls and five during lidocaine exposure) are summarized in Table 2.

In the absence of drug, there were on average the same or fewer openings during the 20-msec pulse for voltage steps to -40 or -50 mV. For voltage steps to -60 and -70 mV, there were fewer openings during the 20-msec pulse. These potentials (-60 and -70 mV) are close to threshold, and the decreased number of events in the 20-msec pulse reflected the long latency to first opening. The steps to -40 and -50 mV minimized the effect of latency to first opening. The findings at -40 and -50 mV during lidocaine exposure contrast with those dur-

ing control. For the experiment illustrated in Figure 4, there was, on average, one opening during the initial 200-msec pulse sequence. During the subsequent sequence of 20-msec pulses, there were, on average, 1.8 openings per pulse. There were 0.7 openings per step during the final 200-msec sequence. The decline in the average number of openings was associated with a marked increase in the number of nulls. The 200-msec steps contained 55% and 63% null sweeps for the initial and final series of steps to -40 mV. On the other hand, the group of 20-msec sweeps contained 29% nulls. In all five experiments with a test potential of -40 mV, fewer openings were observed during the 200-msec pulses compared with the 20-msec pulse. This was also true in three of four experiments at a test potential of -50 mV.

For voltage steps to -40 mV, 2–16% of openings occurred with latencies greater than 20 msec. The number of events occurring at late times (>20-msec latency) were too few to provide a meaningful test of the relation between latency to opening and open times. It is noteworthy that the overall open times are not significantly reduced by lidocaine. If the voltage steps during the 200-msec steps are segregated according to those with and without late events, the steps without late events are still on an average associated with fewer openings and more nulls when compared with the 20-msec step. This suggests that the greater block with the 200-msec pulses is not the result of abbreviation of late openings by blocking events. The block may depend on drug interaction with some channel conformation other than the open state.

Support for the above conclusion is provided from the pulse-train experiment shown in panel C of Figure 4. Trains of ten 200-msec pulses are applied from a holding potential of -120 mV to a test potential of -40 mV during exposure to 80 μM lidocaine. Use-dependent decrease in the number of

TABLE 2. Comparison of Lidocaine Action During 200-msec and 20-msec Pulses

Test potential	Pulse duration (msec)	Average number of openings	
		Control	Lidocaine
-70 mV	200	0.58±0.37	0.2±0.14
	20	0.2±0.2	0.15±0.15
	200	0.6±0.4	0.17±0.12
-60 mV	200	1.6±0.3	0.57±0.12
	20	0.6±0.3	0.33±0.13
	200	1.37±0.3	0.44±0.28
-50 mV	200	1.6±0.4	0.59±0.48
	20	1.4±0.5	0.54±0.22
	200	1.6±0.26	0.4±0.2
-40 mV	200	1.45±0.1	0.42±0.17*
	20	1.16±0.3	0.67±0.23
	200	1.5	0.34±0.2

Values are mean±SD for average number of openings.

\**p*<0.05 (sign test from Reference 27).



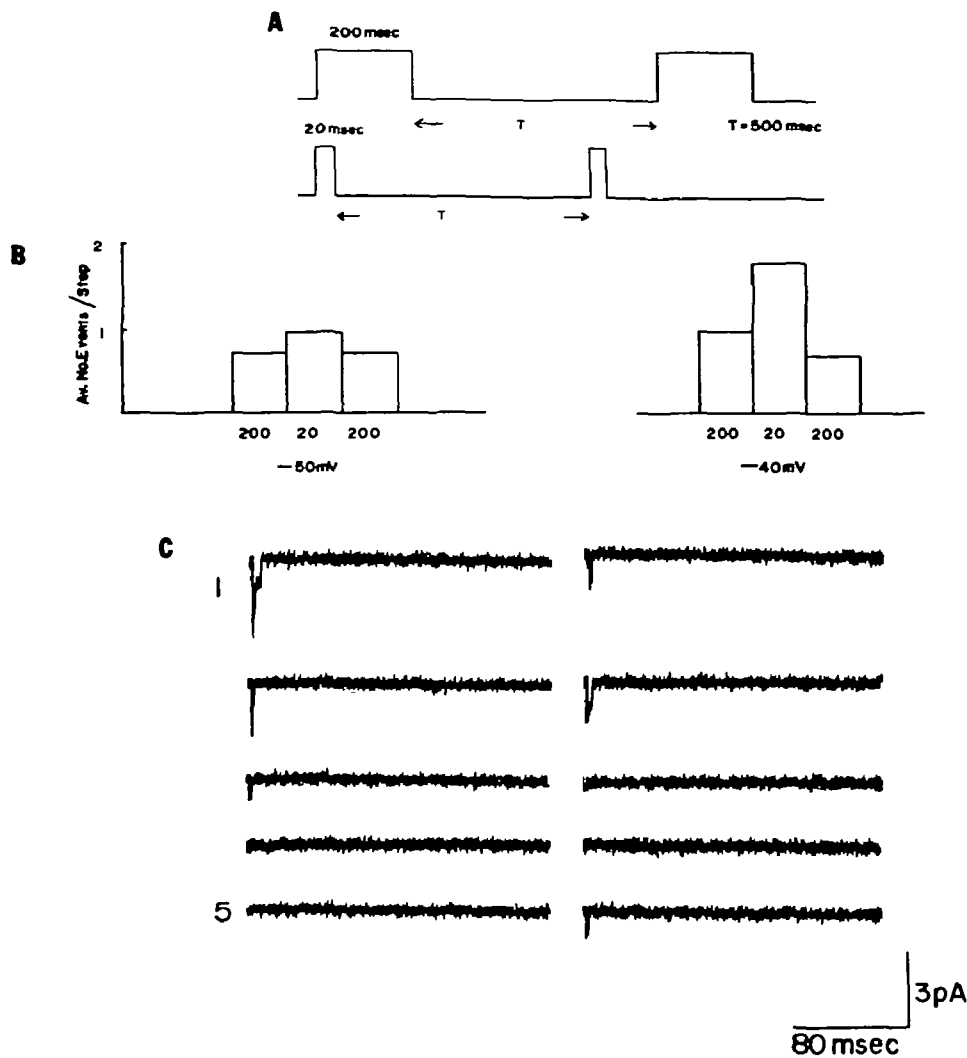


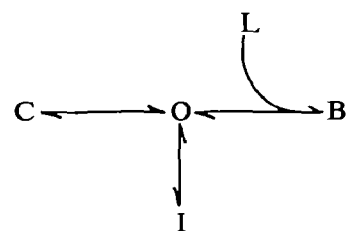
FIGURE 4. Diagrams and tracings showing dependence of opening frequency on pulse duration. Panel A: Pulse paradigm. A set of 200-msec pulses with 500 msec between the pulses is applied initially. These are followed by a set of 20-msec pulses with 500 msec between the pulses. The set of 200-msec pulses are then repeated. Panel B: Average number of openings per step for one experiment for test potentials of  $-50$  and  $-40$  mV. The lidocaine concentration was  $80 \mu\text{M}$ . Panel C: Ten consecutive pulses during a train. The protocol is similar to that shown in Figure 3, but the test potential was  $-40$  mV. Use-dependent block is evident between the first and the tenth pulse in the train. The lidocaine concentration was also  $80 \mu\text{M}$ .

openings is evident from the 10 consecutive pulses shown. The latest channel closure observed in the sequence was 6 msec after the onset of the depolarizing pulse. For 80 trains, the fraction of openings occurring with a latency greater than 20 msec was  $0.9 \pm 1.1\%$ . There is a 71% decline in the average number of openings per pulse between the first and the tenth pulse ( $2.28 - 0.65$ ). If one makes the conservative assumption that the 1% of late-opening channels are all blocked and do not recover between pulses, 71% block could not be observed with 10 pulses. The results support the idea of blockade of a nonconducting channel state.

#### Effects of Lidocaine and RAD-242 on Open-Channel Kinetics

Inasmuch as there are fewer open than closed states of the sodium channel and patches almost invariably contain more than one channel, analysis of open-channel kinetics is potentially the most informative for the sodium channel. The change in open time in the presence of drug provides a direct

measure of the association rate constant of the drug with the sodium channel:



where C, O, B, and I are the resting, open, blocked, and inactivated channels, respectively, and L is lidocaine.

We performed three types of experiments to examine the effect of lidocaine on open-time duration. We compared open time during a prolonged observation period with that observed during the subsequent exposure of the cell to lidocaine. In this type of experiment, no lidocaine is present in the micropipette; however, the hydrophobic drug can diffuse across the cell membrane (e.g., see Reference 14). An example of one such experiment is

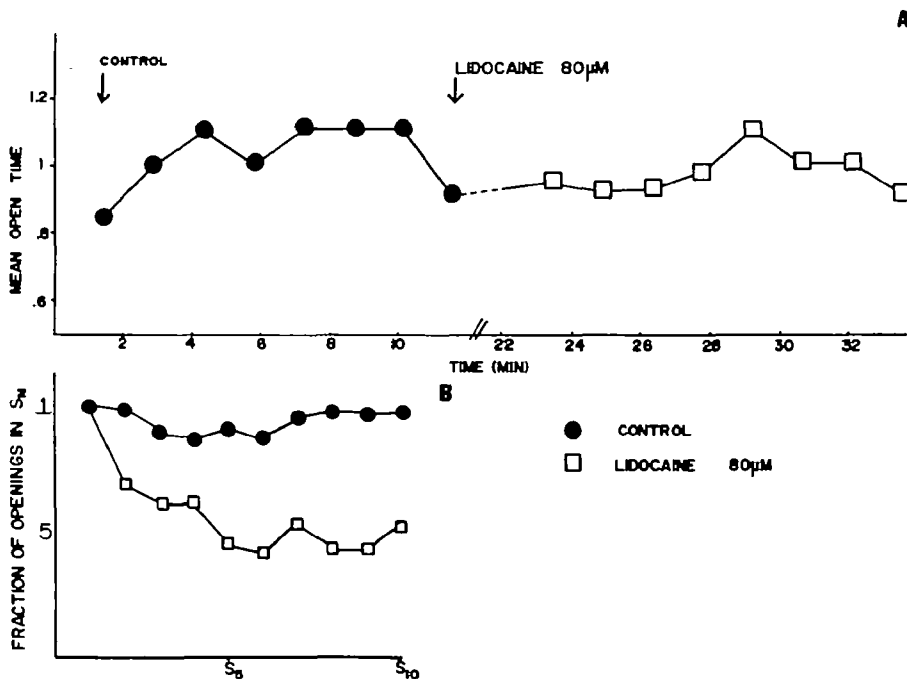


FIGURE 5. Graphs showing effect of lidocaine on single-channel open times. The data illustrated in this figure were obtained during continuous recording from a cell-attached membrane patch. Panel A: Data points that are obtained from groups of 100 voltage steps. After an initial control period, the perfusate was changed to one containing 80  $\mu$ M lidocaine. After a 10-min exposure to lidocaine, the series of voltage steps was repeated. Panel B: Number of openings in each group, which has been normalized by that in  $S_1$  and plotted against the sequence of the pulses within the train (. . .  $S_9$ , . . .  $S_{10}$ ; nomenclature as in Figure 3).

shown in panel A of Figure 5. Eighty trains of 200-msec voltage steps to  $-50$  mV were applied in the absence of drug. Mean channel open time varied from 0.83 to 1.1 msec (panel A). There was no systematic change in the number of openings per channel between the groups of the first and subsequent pulses in the train (panel B). The preparation was then exposed to 80  $\mu$ M lidocaine for 10 minutes, and the trains of pulses were repeated. Mean open times varied from 0.9 to 1.1 msec after lidocaine exposure. There was a progressive decline in the number of openings during the pulse train (panel B). The major cause for the decline in current during use-dependent block is, therefore, a decline in the number rather than the duration of individual events.

The second type of experiment was performed on an excised inside-out patch (Figure 6). Three hundred voltage steps from  $-120$  to  $-60$  mV were applied. The mean open time for the 652 events observed was 2.3 msec. A single exponent provided a good fit to the open-time distribution ( $1/\lambda=2.27$  msec,  $p=0.3$ ; from a minimum  $\chi^2$  procedure). Three hundred seven voltage steps to the same potential were applied after exposure of the membrane patch to 160  $\mu$ M lidocaine. There were a total of 218 events during these steps. The fraction of null sweeps increased from 17% to 60%. The null sweeps tended to occur in runs of as many as 12. The 10 consecutive sweeps shown in panel B of Figure 6 were selected to show events. The mean open time during lidocaine exposure was 2.7 msec. A single exponent did not provide as good a fit to the distribution of open times ( $1/\lambda=2.94$  msec,  $p=0.05$ ). The concentration of lidocaine used in this experiment was 32 times greater than the concentration that produced a 50% reduction in open times in the

experiments of Nilius et al.<sup>13</sup> Possible reasons for this dramatic difference in sensitivity are deferred to "Discussion."

The experiments performed to assess block during pulse trains and during 200- and 20-msec pulses were performed on pairs of patches. Lidocaine was present in the micropipette in one experiment of each pair. These experiments provide a third opportunity to assess the effect of lidocaine on single-channel open time. To assess the variability of mean open time between patches, each pair was stepped to the same potential. We measured open times in three pairs of patches, none of which was exposed to lidocaine. The results are summarized in Table 3A. The variability of open times within pairs was no larger than the variability that may occur with time during continuous measurements in a single patch. Therefore, it seemed reasonable to compare open times at the same potentials in different pairs of patches. For a test potential of  $-60$  mV, we had data from six pairs of patches for comparison of open times. This potential is the same as that used in most of the studies reported by Nilius et al.<sup>13</sup> At other test potentials, there were insufficient pairs for comparison. The data for the test potential of  $-60$  mV are summarized in Table 3B. There was a trend toward a decrease in mean open time; however, this did not reach statistical significance. If a blocker has a weak affinity for the open channel, open-state block may be evidenced by a change in conductance. We were able to measure the conductance in three pairs of patches in which the events from at least 80 steps were available at three or more potentials. The mean conductance was 20 pS during control and 18 pS during lidocaine exposure.

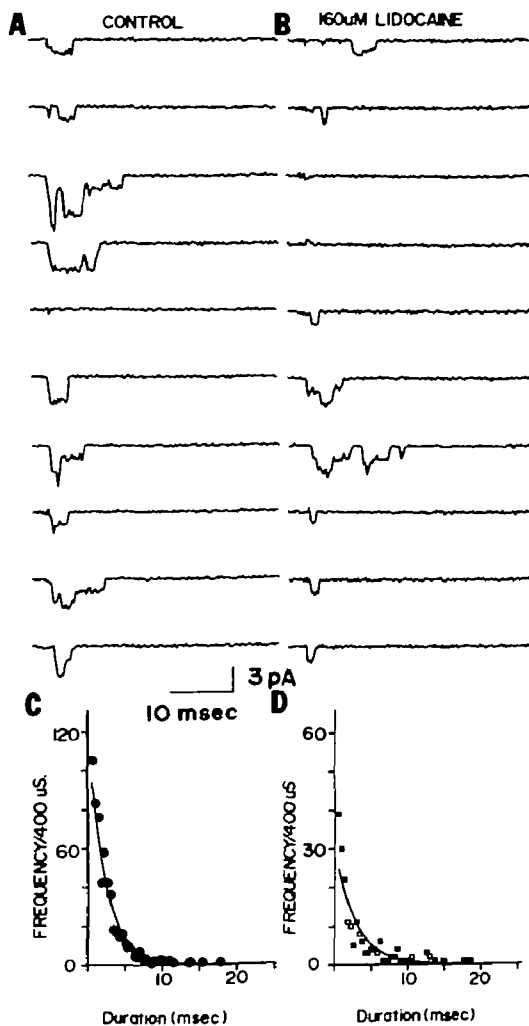


FIGURE 6. Tracings and graphs showing effect of lidocaine on single-channel open time in an excised patch. Panels A and B; Ten consecutive steps during control and the subsequent exposure to 160  $\mu$ M lidocaine in an inside-out membrane patch. The holding potential was  $-120$  mV; the voltage step was to  $-60$  mV. Only the first 34 msec of the 200-msec steps are shown. Panel C: Distribution of opening times during control exposure. Panel D: Distribution of opening times during lidocaine exposure. The continuous lines are density functions,  $f(t) = \lambda \exp(-\lambda t)$  that have been fitted to the observations ( $t$ , time;  $\lambda$ , closing rate constant). The actual plots were obtained by multiplying the equation by the expected total number of events. This step is necessary because of the constraint that the area under the probability density curve should be 1. ( $\lambda$  is  $0.44/\text{msec}$  during control and  $0.34/\text{msec}$  during lidocaine exposure.)

In their voltage-clamp experiments in rabbit Purkinje fibers, Bean et al<sup>26</sup> remarked that they could find evidence for open-channel block only when the external pH was raised to 8.1 and the lidocaine concentration increased to 200  $\mu$ M. This suggested that intracellular drug or drug within the membrane may block open channels. Because of the negative

TABLE 3. Comparison of Mean Open Time in Different Patches

Patch	$V_T$ (mV)	Mean open time	
		Control (msec)	Lidocaine (msec)
<i>Without lidocaine</i>			
t,u	-60	$0.8 \pm 1$	...
		$0.8 \pm 0.9$	...
v,w	-80	$1.2 \pm 1.2$	...
		$1 \pm 1$	...
x,y	-60	$1 \pm 1$	...
		$1 \pm 1$	...
<i>With lidocaine</i>			
b,a	-60	$2.3 \pm 2.1$	$1.5 \pm 1.7$
e,f	-60	$1.7 \pm 1.5$	$1.5 \pm 1.7$
g,h	-60	$1.4 \pm 1.4$	$1.1 \pm 1.2$
s,r	-60	$1.5 \pm 1.5$	$1.1 \pm 1.2$
q,p	-60	$0.7 \pm 0.8$	$0.8 \pm 0.8$
d,c	-60	$0.7 \pm 0.8$	$0.9 \pm 0.9$
Mean of six pairs		$1.4 \pm 0.6$	$1.2 \pm 0.3$

Values are mean  $\pm$  SD for mean open time.  $V_T$ , test potential.

results in the inside-out patch described above, we tried another approach. We examined the blocking action of the lidocaine derivative RAD-242. A pentyl group replaces one of the ethyl groups of the tertiary nitrogen atom of lidocaine. The compound has a lipid solubility 10-fold greater than lidocaine and is capable of blocking the nerve action potential.<sup>17</sup> We examined the effects of 40  $\mu$ M RAD-242 on single-channel open time. To establish that this concentration produced at least comparable sodium-channel block to lidocaine, we performed an experiment with 80 trains each of 10 pulses. The results are summarized in Figure 7. The 10 steps in panel A are consecutive. The maximum number of overlapping events was evident during the first pulse. The averaged currents from the groups of first pulses ( $S_1$  in the nomenclature of Figure 3) and the tenth pulses ( $S_{10}$ ) of the trains are shown in panel B. RAD-242 decreased the peak value of the averaged current by 60%. As shown in panel C, the onset of the effect appeared to be of a rate similar to lidocaine and was associated with a substantial increase in the number of null sweeps. We have summarized the results of a pair of patches, one during control and another during exposure to RAD-242, in Table 4. We could not discern any trend for the drug to reduce the mean open time. This suggests that RAD-242 may cause substantial use-dependent block yet produce little change in open times when the activated channels do conduct.

#### Drug Effect on Slow Sodium Channel Kinetics

Single-channel studies by Patlak and Ortiz,<sup>10</sup> Grant and Starmer,<sup>12</sup> and Grant et al<sup>28</sup> have recently identified slow-gating kinetics in a low percentage (<1%) of depolarizing sweeps in ventricular and Purkinje cells. These slow currents may result from

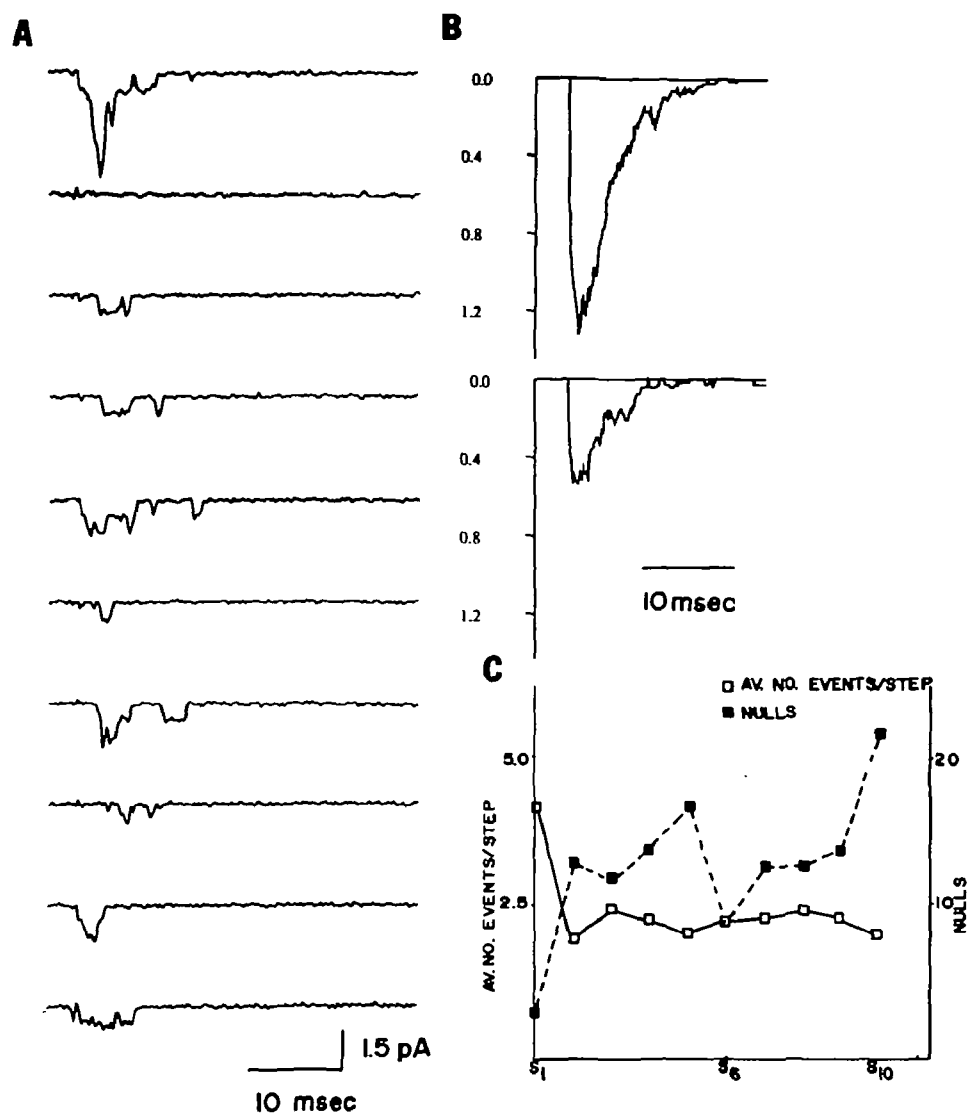


FIGURE 7. Tracings and graph showing block of single cardiac sodium channels by RAD-242. Panel A: Current responses to 10 consecutive steps from  $-120$  to  $-60$  mV during exposure to  $40 \mu\text{M}$  RAD-242. Only the first 34 msec of the 200-msec pulses are shown. Panel B: Averaged current for the first and tenth steps ( $S_1$  and  $S_{10}$  in the nomenclature of Figure 3). The ordinate is in units of the unitary current amplitude ( $1.6 \text{ pA}$ ). Panel C: Average number of events per step and the total number of nulls in each group of 80 steps.

transient failure of inactivation and may account for the tetrodotoxin-sensitive component of the action potential duration. Nilius et al<sup>13</sup> have shown that lidocaine abolishes bursts of late openings in unmodified sodium channels and those induced by aconitine.

Because of the low probability of observing bursts of opening, we were unable to study lidocaine's effects on such bursts before and after drug exposure in the same patch. We can make only qualita-

tive statements about the channels showing slow-gating kinetics. In contrast to the complete abolition of late bursts by  $10 \mu\text{M}$  lidocaine observed in the study of Nilius et al,<sup>13</sup> we saw examples of such late bursts occurring in three patches during exposure to  $80 \mu\text{M}$  lidocaine. Panel A of Figure 8 shows four consecutive steps to  $-50$  mV in a cell-attached membrane patch during exposure to  $80 \mu\text{M}$  lidocaine. The initial surge of sodium current (truncated in the figure) is followed by late openings. In

TABLE 4. Effects of RAD-242 on Single Sodium Channels

Test voltage	Control		RAD-242	
	Mean open time (msec)	Current amplitude (pA)	Mean open time (msec)	Current amplitude (pA)
$-70 \text{ mV}$	$0.8 \pm 0.8$	1.7	$0.8 \pm 0.6$	1.7
$-60 \text{ mV}$	$0.9 \pm 0.9$	1.7	$1.2 \pm 1.2$	1.6
$-50 \text{ mV}$	$1.4 \pm 1.4$	1.5	$1.6 \pm 1.8$	1.4
$-40 \text{ mV}$	$1.7 \pm 1.9$	1.4	$1.4 \pm 1.4$	1.2

Values are mean  $\pm$  SD for mean open time.

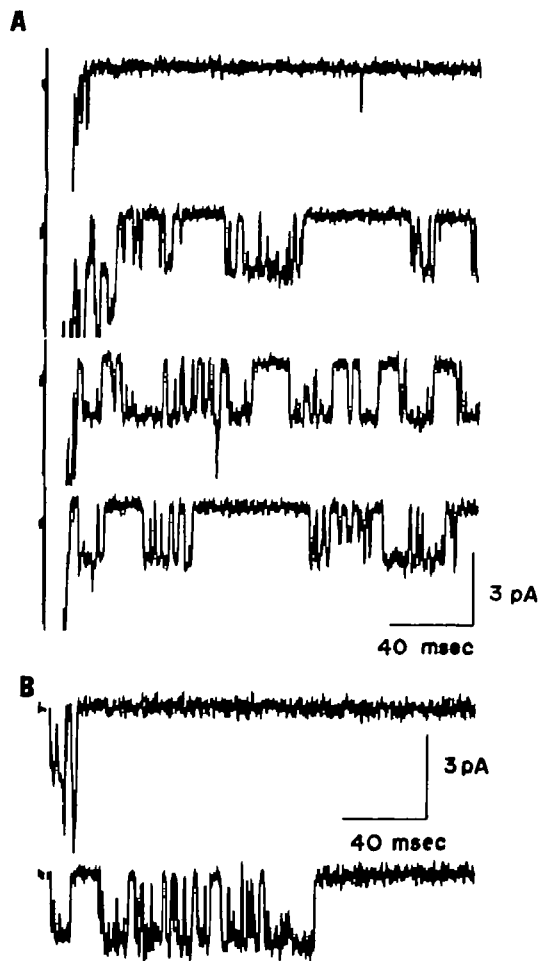


FIGURE 8. Tracings showing slow-gating kinetics during lidocaine exposure. The records in panels A and B were obtained from separate patches during exposure to 80  $\mu\text{M}$  lidocaine. The holding potential was  $-120$  mV. Panel A: Voltage step to  $-50$  mV. Panel B: Voltage step to  $-40$  mV. In panel A, the patch contained at least 20 channels, and the initial surge of current saturated the data logging system. The data in panel B were obtained from a patch with three channels.

the first tracing, the late opening consists of a single brief event. In the second, third, and fourth tracings, bursts of late events are seen throughout the 200-msec duration of each voltage step. Because these are consecutive tracings with an interstimulus interval of 500 msec, it suggests that the rate constant for exit from the slow-gating mode is quite small.<sup>12</sup> The mean shut time was 7.9 msec. However, a single exponent did not provide a good fit. There are clearly long shut intervals between bursts and shorter intervals within bursts. Panel B shows another example of bursts of late opening occurring during lidocaine exposure in a different patch. However, in this example, the bursts only lasted for a total of 117 msec. Comparison of bursts occurring during lidocaine exposure to those in the absence of drug (e.g., panel C of Figure 1) suggests that

lidocaine might increase the interval between bursts or decrease sojourn in the bursting mode. However, because of the heterogeneity of burst kinetics, exact comparisons would require comparison in the same patch.<sup>29</sup> Bursts in the unmodified channel are, therefore, not totally abolished by 80  $\mu\text{M}$  lidocaine, but the kinetics (e.g., shut times between bursts) may be modified to produce a net decrease in late sodium current.

#### Discussion

We have explored the mechanisms of block of single cardiac sodium channels by lidocaine. Our results show that the major mechanism of block during use-dependent inhibition is a reduction in the number of open events. The block that occurs with long pulses does not seem to be dependent on the occurrence of late openings. Within the limits of our experimental error, we could not detect a reduction in mean channel open time. Even with a potent hydrophobic derivative of lidocaine that caused a 60% reduction of peak average current, we could not detect a reduction of mean channel open time. Bursts of late opening persist in the presence of lidocaine. However, the kinetics (e.g., interval between bursts) may be modified to produce the reduction in late current during lidocaine exposure. We shall first review some of the important limitations of this study.

The studies were performed with a free drug concentration of 80  $\mu\text{M}$  or more. This drug concentration is clearly in toxic concentration range for clinical arrhythmia suppression.<sup>30</sup> However, this was a compromise choice. Because of the stochastic nature of single channel events, the gathering of statistically meaningful data requires a large number of repetitions of any one voltage protocol. The time period over which stable recordings can be obtained is relatively brief. We used large drug concentrations to be certain that a drug effect could be readily established. It is fully recognized that new blocking mechanisms may become evident with the high concentrations we used.<sup>31</sup> However, these concentrations should provide insight into the spectrum of blocking effects that the drug may exhibit.

We observed significant variation in channel kinetics with time in the absence of drug exposure. When we initiated these studies, we made most of our recordings in excised patches. However, the studies were often complicated by rundown. The choice of the principal cation (potassium, cesium, or *N*-methyl-D-glucamine), anion (chloride, fluoride, or sulfate), or the presence of ATP did not prevent an unpredictable rundown. Horn and Vandenberg<sup>16</sup> have suggested that time-dependent change is more common with excised than cell-attached patches. Therefore, we chose to do the major portions of this study in cell-attached patches. Even with this recording configuration, channel kinetics were not always stable. We seriously doubt that the problems were

unique to our experimental setup. Other investigators have commented on the need to collect the data in blocks to identify change in kinetics or have pointed out the occurrence of apparent shifts in channel gating even in cell-attached patches.<sup>10,11,23</sup> The changes can quite easily confound analysis of drug effects. For example, time-dependent rundown could lead to abnormally low  $K_d$  for drug blockade and almost certainly prevent the performance of cumulative dose-response curves. As far as possible, we tried to design protocols that were not critically dependent on long-term stability of the kinetics.

Use-dependent block of sodium channel was associated with a decline in the number of openings during successive steps during trains (i.e., a reduction in  $P_o$ ). In their study of the blocking action of amiodarone, propafenone, and diprafenone, Kohlhardt and Fichtner<sup>32</sup> observed a decrease in the number of openings of sodium channels as stimulation frequency was increased. We were unable to detect a significant shortening of the mean open time during the train. This suggests that a blocking event may not be important in terminating the open state. The same processes, deactivation and inactivation, may remain the principal channel closure mechanisms even in the presence of lidocaine. The lack of an effect on mean open time is at variance with the results of Nilius et al.<sup>13</sup> They observed a 57% reduction of mean open time during exposure to 5  $\mu$ M lidocaine in inside-out patches of guinea pig ventricular myocytes. We observed no effect on open time with 80–160  $\mu$ M lidocaine in rabbit ventricular myocytes. The high potency observed by Nilius et al does not agree with the experiments on the macroscopic sodium current in multicellular and isolated myocyte preparations. Thus, Bean et al<sup>26</sup> had to use lidocaine concentrations of 200  $\mu$ M coupled with a pH of 8.1 to demonstrate open-channel block. They demonstrated a rapid phase of block with brief (10-msec) conditioning pulses to threshold but not to subthreshold potentials. These experiments were performed under conditions of reduced extracellular sodium concentration. The single-channel experiments have the advantage that potential open-channel blocking effects can be examined in normal extracellular sodium concentration. Experiments in nerve suggest that open-channel block is antagonized by external sodium ions.<sup>33</sup> Kohlhardt et al<sup>15</sup> did not observe any effects of lidocaine on the open time in inside-out patches of rat neonatal myocytes. Like lidocaine, amiodarone blocks the sodium channel with a relatively slow time course over the duration of the normal action potential. Kohlhardt and Fichtner<sup>32</sup> could demonstrate no effect of amiodarone on single-sodium channel open time at drug concentrations that caused prominent frequency-dependent inhibition. Zaborovskaya and Khodorov<sup>34</sup> removed inactivation in the sodium channel of myelinated nerve with chloramine T. Lidocaine (0.1–1 mM) did not have any effect on the peak amplitude of

the noninactivating component of the current. This suggests that the drug does not block open sodium channels in nerve.

In the acetylcholine-activated channels in denervated skeletal muscle and in the sodium channel with inactivation removed, the following simple sequential model accounts for the blocking action observed at the single channel level<sup>35</sup>:



where C, O, and B are the closed, open, and blocked states of the channel, respectively, [D] is drug concentration and  $k_1$  is the respective rate constant. It should be pointed out that in such a scheme, the number of events should increase in the presence of a blocker. The drugs used in those studies, QX222, QX314, and 9-aminoacridine, have large dissociation rate constants and dissociate from the channel before the latter finally closes.<sup>35,36</sup> A single opening in the absence of drug was converted to a burst of openings during drug exposure. More importantly, the total open time should be unchanged. Neher and Steinbach<sup>35</sup> suggested that a similar mechanism may account for the block of sodium channels by local anesthetics. Our results do not support that suggestion. We could not identify an important component of open-channel block by lidocaine. On the contrary, our results show that a significant amount of block continues during long pulses after the end of the initial sodium current transient.

We were interested in how the failure to show an effect on channel open time may be related to fast closure of the normal channel. The lack of any observed drug effect on channel open time could be a technical problem. In a variety of tissues, a number of drugs block ionic channels with association rate constants of the order of  $10^6$ – $10^7$ /M/sec (see reference 35). If one assumes a rate constant of  $10^7$ /M/sec for lidocaine, the blocking rate in the presence of 80  $\mu$ M lidocaine should be  $\sim 0.8$ /msec. The closing rate of the cardiac sodium channel during control under our recording conditions was 0.5–1/msec. One would expect to observe a 30–50% reduction in open times. Such differences should be resolvable. They are clearly larger than the variation in open times that we observed between pairs of patches. On the other hand, if one assumes a blocking rate constant of  $10^6$ /M/sec, the reduction of open time would be 10%. Such a relatively small change in open time could well have been missed in the current experiments. The fact that such a change would be small should not detract from the potential importance of this mechanism. Experiments with inactivation modifiers may help to resolve this question. Another approach would be to increase the binding rate by increasing drug concentration. However, the drug concentrations used are already in the toxic range. We should also emphasize that our

experiments were carried out over a very limited range of potentials. The decrease in conductance as the sodium reversal potential is approached does not permit acceptable resolution of single-channel currents in the positive range of potential with normal sodium gradients. The +20 to +40 mV range of potentials may be important because peak depolarizations during the action potential occur at positive potentials. If open-channel blockade is voltage dependent, we could have failed to demonstrate an effect on open because of the limited voltage range studied.

Nilius et al<sup>13</sup> also reported a curious drug effect on open times. They pointed out that hyperpolarization relieved the block produced by lidocaine and restored open times toward normal. In the absence of drug, mean channel open time depends on test potential only and not on the holding potential.<sup>23</sup> In the presence of a blocker, open events are terminated by normal closure or by blockade. As pointed out by Colquhoun and Hawkes ("the unblocked channel fallacy"),<sup>37</sup> open events that are terminated by either process should have the same duration distribution. Therefore, one would not expect to see any change in open times when block is partially relieved by hyperpolarization.

We summarize what we consider potentially important differences in the recording conditions and discuss the extent to which these are likely to account for the observed differences between the observations of Nilius et al<sup>13</sup> and our own. Their studies were performed in guinea pig ventricular myocytes; our own were performed in rabbit ventricular myocytes. We are not aware of any data showing marked species differences in the sensitivity to the blocking action of lidocaine. They used 2.5 mM calcium in the pipette solution whereas we used 0.02 mM; we used such a low concentration to obtain a linear current-voltage relation. Upstroke velocity measurements in atrial myocardium suggest that lidocaine is more depressant in 1.25 mM than in 5 mM extracellular calcium concentration.<sup>38</sup> There was a 2° C difference in the temperature at which the recordings were made. This does not appear likely to account for the differences in results. We see no likely methodical reason for the difference in results other than the choice of animal species.

Our results show that lidocaine produces greater block during 200-msec pulses compared with 20-msec pulses. This is consistent with the slow time of block observed in macroscopic current experiments. Thus Sanchez-Chapula et al<sup>25</sup> observed a half time for blocking of 115 msec at -30 mV. In the study of Bean et al,<sup>26</sup> block developed over a time frame of several seconds. This has been interpreted as evidence for drug binding to the inactivated state of the sodium channel.<sup>25,26,39</sup> Our single-channel results are consistent with this conclusion. The block occurring at late times does not appear to depend on the abbreviation of late openings. Two

hundred-millisecond pulses with no events occurring after 20 msec are associated with greater block than shorter pulses of 20-msec total duration. Our experiments do not address whether the binding is occurring to the inactivated state of the channel or to some channel conformation having a similar voltage dependence.

Lidocaine decreases action potential duration in both Purkinje and ventricular cells.<sup>40</sup> This effect is more prominent in the former cell type. Voltage-clamp experiments in rabbit and sheep Purkinje fibers show that lidocaine shifts the background current-voltage relation in the outward direction.<sup>41-43</sup> However, no outward shift is observed with prior exposure to tetrodotoxin. The data suggest that lidocaine blocks a slow component of tetrodotoxin-sensitive current. Similar results have recently been obtained in dog ventricular myocytes by Wasserstrom and Salata.<sup>44</sup> Single-channel studies by Patlak and Ortiz,<sup>10</sup> Grant and Starmer,<sup>12</sup> and Grant et al<sup>28</sup> have recently identified a slow component of sodium current in ventricular myocytes and Purkinje cells. Nilius et al<sup>13</sup> reported that this component of slow current was abolished by 5 μM lidocaine. We continued to observe occasional pulses showing slow kinetics during lidocaine exposure. Therefore, the action of the drug in shortening the action potential may not be related to abolishing slow gating. Rather it may result from a change in such kinetics, such as an increase in shut intervals between or within burst. Because of the heterogeneity in burst kinetics, quantitative analysis would require experiments performed on the same patch during control and lidocaine exposure. The phenomenon of slow gating is rare. We were unable to make such observations in the same patch.

In summary, the most prominent effect of lidocaine we observed was a failure of the channel to open during use-dependent inhibition. We could not demonstrate an effect on mean open time. Prolongation of the depolarizing pulse enhanced block even when no events were observed at late times during the prolonged pulse. This suggests blockade of nonconducting state(s) of the channel. Inasmuch as all our patches contain multiple channels, it was not possible to do meaningful quantitative analysis on shut times or distribution of null sweeps.<sup>45</sup> The principal blocking action of lidocaine on the sodium channel is similar to the blocking action that has been reported for dihydropyridine derivatives in single calcium channels.<sup>45-48</sup>

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